differentiation. The gene expression of *COL2*, *SOX9* and *ACAN* increased in hBMSC pellets treated with Notch1-antagoistic aptamer for both of 7 (0 to 7) and 14 (0 to 14) days compared to those of untreated hBMSCs pellets. The pellets treated with Notch1-antagonistic aptamer showed greater intensity of safranin-O staining than those of untreated BMSCs. However, there was no synergistic effect of Notch1-antagonistic aptamer with TGF- β in chondrogenic differentiation of hBMSCs pellets. 5) Gene expression profiling with oligonucleotide microarrays. We selected 82 chondrogenesis-related genes from microarray data and then hierarchical clustering of selected probes was performed. Different patterns were detectable between Notch1-antagonistic aptamer and untreated control.
Conclusion: We investigated the Notch1-antagoistic aptamer as a candidate of nucleic acid therapeutics to induce chondrogenic differentiation of hBMSCs. Selected Notch1-antagonistic aptamer induced complete chondrogenesis in hBMSCs pellets even though early treatment (0 to 7 days).
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Session: Imaging (Diagnosis & Treatment) — (Micro)CT

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DIFFERENT THRESHOLD TO ANALYZE THE OSTEOGENESIS RESULT BY MICRO CT

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Objective: To evaluate the influence of different threshold in micro-CT on the result of bioactive glass implanted into rabbit, and to find the effective method to assess the osteogenesis effects by micro-CT.
Methods: We prepared the New-Zealand white rabbit femoral condyle defect model with bioactive glass embedded in defect parts, and took the bioactive glass out after three months. Using different threshold range to represent the new bone and the remaining material respectively, the bioactive glass was scanned by the micro-CT to analyze the new bone volume fraction and the percentage of the rest material among different groups. We made the decalcified biopsy of the bioactive glass, Van Gieson staining was to assess the new bone formation percentage of bioactive glass by Image-Pro Plus software.
Results: The new bone volume fraction of 1 and 2 group was 20.36±1.657% and 22.04±2.220%, which conformed to the result of Van Gieson staining (21.33±1.250%) reasonably.
Conclusion: There were large differences of bioactive glass osteogenesis ability by different threshold analysis of micro CT, and it need to be combined with histopathological findings to contrastive analysis to find a proper threshold.

Table 1 Threshold segmentation scope of each group.

	A	B	C	D	E	F
Bone tissue	1000—2500	1100—2600	1200—2700	1300—2800	1400—2900	1500—3000
Remaining material	≥2500	≥2600	≥2700	≥2800	≥2900	≥3000

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Session: Biomechanics — Joint Kinematics

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KINEMATIC ANALYSIS OF ANKLE EVERSION SPRAIN IN SPORTS: TWO CASES DURING THE FIFA WORLD CUP

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Background: The ankle is one of the most commonly injured joints with sprains. In the all ankle sprains, nearly 85% are lateral sprains, while, medial sprains, which also known as the eversion ankle sprain, are less common than lateral ankle sprains. However, medial sprains are typically associated with subsequent syndesmosis injury or medial malleolus fracture, and represent a more disabling problem, requiring longer recovery and different treatment. A precise description of the injury situation is a key component to understanding the injury mechanism and preventing injuries. However, quantitative analyses on injury cases with calibrated video recording are available only under rare circumstances. Instead, injuries in sports are occasionally shown on television with multiple camera views, by using a novel biomechanical motion analysis technique, model-based image matching (MBIM), those videos could be further analyzed. The purpose of this study was to present the 3-dimensional ankle joint kinematics of two ankle eversion sprain cases.
Subjects and Methods: Videos (50 Hz) that recorded the two injury cases were obtained from the 2010 FIFA Broadcasting System, captured by two video cameras for case 1 and three cameras for case 2. The videos were transformed into image sequences, then synchronized and rendered into 1-Hz video sequences. The matching procedure was performed using 3-D animation software Poser 4 and Poser Pro Pack (Curious Labs Inc, Santa Cruz, California) with a skeleton model. The skeleton matching started with the shank segment and then distally matched the foot and toe segments frame by frame. The joint angle time histories were read into Matlab with a customized script for data processing. Ankle joint kinematics results were filtered with 15-Hz cut-off frequency.
Results: The injury occurred when the players were striving for the ball both in case 1 and case 2. The players were trampled by their opponents on the lateral of lower leg and suffered a severe eversion ankle sprain. At the point of trampling, the ankle joint everted 3.0°, externally rotated 8.0°, and dorsiflexed 30.0° in case 1. In case 2, it inverted 1.1°, externally rotated 3.6°, and plantarflexed 0.3°. At 0.20s in case 1 and 0.22s in case 2 after trampled, the eversion angle reached the maximum with the ankle joint 25.2° and 20.0° everted, 42.3° and 49.4° externally rotated, 15.0° and 30.6° plantarflexed respectively. The maximum eversion velocity was 210°/s and 320°/s.
Discussion and conclusion: Compared with the inversion cases that had been reported, both the maximum angle and maximum velocity of eversion are obviously lower. It is due to the biomechanics of the ankle joint, which allow for less eversion than inversion. Also the deltoid ligaments are strong and often an avulsion fracture at the medial malleolus occurs before a deltoid ligament sprain. The results of this study also suggested that the method and equipment designing to prevent eversion ankle sprain should be different from that to prevent inversion ankle sprain. The results from the MBIM technique could contribute to the understanding of the injury mechanism of ankle sprain injury.
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Session: Clinical Studies

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ALTERATION OF CYTOKINES EXPRESSION AND FUNCTION IN LIPOPOLYSACCHARIDE-INDUCED RAW264.7 CELLS UNDER SIMULATED MICROGRAVITY CONDITION

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Objective: Macrophages play a vital role in the innate immune system. Lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, stimulates immune responses to induce the generation of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6. The mechanism by which the LPS signal is transduced from the extracellular environment to the nuclear compartment is not well defined. There are a lot of studies have demonstrated that microgravity is one of the main reasons causing immune disorder, especially the change of macrophage function in space. Therefore, the purpose of present investigation is to elucidate the effects and possible mechanism of macrophage activation induced by the LPS. Furthermore, whether the activation and function of macrophage can be improved by LPS under the condition of simulated microgravity (SMG).
Materials and Methods: Murine RAW264.7 cells were generally cultured in α-MEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 1μg/ml LPS at 37 °C. Under the simulated microgravity condition(SMG), RAW264.7 cells were seeded in carrier slides, after 24h growth, the carrier slides were put in a revolving boxes, which was filled with medium, tightly capped, and were fixed onto the Random-positioning machine (RPM). RPM was rotated randomly at the range of 0～10 rpm and was located in an incubator providing 37°C. Control groups were also completely filled up with medium and cultured in the incubator at 37°C. After 4h, the cell were collected for the functional experiments and further study. To confirm the activation of macrophages by LPS, quantitative RT-PCR analysis was further performed to analyze the mRNA levels of iNOS, TNF-α, IL-1β, IL-6 and IL-10 in macrophages. In addition, the phagocytosis of macrophages were tested.
Results: After culturing 4 h under SMG, LPS-induced TNF-α and IL-10 expression in mouse macrophages were significantly suppressed. However, the mRNA expression of iNOS and TLR-4 were increased markedly. TNF-α, IL-6, IL-1β and NO secretion by macrophages in the culture supernatant were further determined. Less IL-6 and IL-1β was secreted by the LPS-stimulated macrophages under SMG, compared with those macrophages under normal gravity. But there was no difference about the secretion of TNF-α and NO. In addition, the phagocytosis of macrophages were decreased significantly in SMG.

Discussion and Conclusions: Short-term treatment with microgravity caused significantly changed of inflammatory cytolines production. The differential effects of simulated microgravity on TNF-α, IL-6, IL-1β, TLR-4 expression suggested the different sensitivity to microgravity of signaling pathways regulating different inflammatory cytokines in macrophages. Furthermore, microgravity impaired the function of macrophage. The decreased inflammatory response of macrophages and the damaged function caused by microgravity may contribute to the increased susceptibility to infections of astronauts. LPS stimulates immune responses by interacting with the membrane receptor TLR-4 to induce the generation of cytokines. In the future work, the LPS-induced activation of signaling pathways downstream of TLR4, such as NF-κB and MAPK pathways will be tested.
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Session: Disease & Treatment — Tumors

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Mfn2 INHIBITS THE PROLIFERATION OF OSTEOSARCOMA VIA DOWNREGULATION OF plk1

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Objective: The tumor suppressor role of mitochondrial fusion gene 2 (Mfn2) have been confirmed in many tumors. However, there are few reports about the mechanism of mfn2 on the development and progression of tumor. We will study the osteosarcoma in this research to reveal the correlation of Mfn2 and osteosarcoma. A further demonstration of the potential mechanism between them will be included.
Methods: We collect the pathological specimens of osteosarcoma patients, and then analysis the expression of mfn2 and its effect on the survival of patients with osteosarcoma. A construction of lentiviral which overexpress the mfn2 is needed. We would infect the osteosarcoma cell lines MG63 and U2OS with the lentiviral and then selected the positive cells with puromycin to obtain the stable line. The exploration of the overexpression of mfn2 on the proliferation and the apoptosis will be carried out. The purification of flag-mfn2 and a mass spectrometry analysis are necessary to detect the interaction protein of mfn2. What's more, the interaction will be further identified through the immunoprecipitation (IP). The knock out or knock down of mfn2 in the osteosarcoma cell through the CRISPR/cas9 are vital important to our mechanism analysis. After the above clinical research and cell based experiments, we would like to construct an mfn2 knock in mouse model and induce the development of osteosarcoma. We will study the effect of mfn2 on the development and the progression of osteosarcoma and the potential mechanism, to provide a research foundation for precision treatment.
Results: (1) The expression of mfn2 is lower in osteosarcoma tissue than the adjacent non tumor tissue both in mRNA and protein level. And the low expression of mfn2 is corrected with the poor prognosis. (2) We are the first time identified the plk1 has an interaction with mfn2 through mass spectrometry analysis. The interaction between mfn2 and plk1 has been further confirmed through the IP. (3) The stable line U2OS in which the mfn2 is knocked down has been obtained by the CRISPR-cas9 technique. (4) There has a negative relationship between the expression level of mfn2 and plk1.
Conclusion: mfn2 is a novel tumors pressor for osteosarcoma. And it functions its tumor inhibition roles through the negative regulation of the expression level of plk1.
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Session: Others

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RESVERATROL ATTENUATES OXIDATIVE STRESS INDUCED UP-REGULATION OF P-SELECTIN, PSGL-1, vWF AND TM VIA SIRT1 PATHWAY IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Objective: To investigate the effects and underlying mechanisms of resveratrol on pro-thrombosis factors in human umbilical vein endothelial cells under oxidative stress injury.
Methods: Construct H_2O_2 induced HUVEC cells injury model in vitro, inspecting PI3-K/Akt/GSK3β, MAPKs, NF-κB and Nfr2/ARE signaling pathway in OSI mediated endothelial cells injury and apoptosis, exploring effects and molecular mechanism of resveratrol protecting venous endothelial cells from oxidative injury and apoptosis. By overexpression and inhibition of SIRT1, explore regulation effects of resveratrol on the secretions of pro-thrombotic molecular—P-Selectin, P-selectin glycoprotein ligand-1 (PSGL-1), thrombomodulin (TM) and von Willebrand factor (vWF) in HUVECs and downstream key signaling pathways.
Results: PI3-K/Akt/GSK3β, MAPKs (c-Jun, ERK1/2, p38), NF-κB, Nfr2/ARE signaling pathway are involved in venous endothelial cells apoptosis induced by oxidative stress. Resveratrol could inhibit pro-apoptotic pathway (MAPKs), inhibit inflammatory response pathway (NF kappaB), up-regulate anti-apoptotic pathway (PI3-K/Akt/GSK3β), protecting endothelial cells from oxidative stress induced apoptosis and injury. Resveratrol could inhibit P-Selectin, PSGL-1, vWF and mRNA TM and protein expression in oxidative induced endothelial cells via SIRT1 pathway.
Conclusion: Resveratrol could protect endothelial cells from oxidative induced apoptosis and injury, inhibit pro-thrombosis molecules secretion, suggesting a new target for drug prevention and treatment of deep venous thrombosis.
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Session: Traumatology — Fixation

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SINGLE LOCKING PLATE CONSTRUCTS ARE LESS SENSITIVE TO SCREW REMOVAL THAN DUAL LOCKING PLATE CONSTRUCTS FOR MID-DIAPHYSEAL FRACTURE FIXATION

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Background: Single large-fragment plate constructs are currently the norm for internal fixation of mid-diaphyseal humerus fractures. In cases where anatomy limits the size of the humerus available for fixation however, recent studies support the use of a dual small fragment locking plate construct. This study aims to compare the simulated performance of both a single locking plate and a dual-locking plate construct with decreasing number of locking screws and with changes in screw fixation location.
Subjects and Methods: Mid-diaphyseal humeral fracture fixation using a single (Model S) and a dual (Model D) locking plate construct were simulated using the finite element method, a numerical technique commonly used to computationally approximate solutions for complex structural mechanics problems. Different configurations were tested by removal of either one or two screws from the superior half of the fixation construct and compared to a control having no screws removed. Models are labelled based on the location of the screw removed with 1 denoting the most superior screw and 4 denoting the inferior screw adjacent to the fracture (e.g., S1 denotes removal of the most superior screw from the single plate model).
Results: Model D4 was the only construct to show an increase in stiffness as compared to the original dual plate construct without any screws removed. For the single-plate constructs, models S1-S3 all resulted in less than 2.5% stiffness reductions as compared to the control. Noteworthy, three of the single plate constructs, having two screws removed (models S12, S13, and S23), showed less than a 6% reduction in construct stiffness. In contrast, all of the dual-plate constructs with 2 screws removed showed high stiffness reductions (greater than 55%).
Discussion and Conclusion: Results support that screw number and/or location and construct type (single vs dual) are important factors to consider in achieving successful fixation. Based on the simulations performed, the single plate models were found to be less sensitive to screw removal. A balance must be achieved between hardware (i.e., screws and plate) stresses and construct stiffness. Increased hardware stress can lead to early failure while changes in construct stiffness may affect the ability for bone to heal. Future experimental and clinical studies are needed for surgical recommendations especially with regards to the relationship between our outcome measures and healing based on interfragmentary motion.
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Session: Biomaterials and Implants — Bioinert/Bioactive Materials

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***IN VITRO* AND *IN VIVO* EVALUATION OF MACRO-PORE BIOGLASS BONE BLOCKS AND THE APPLICATION IN LOAD-BEARING DEFECTIVE BONE**

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Seeking for a kind of suitable bone biomaterials to repair the heavy defective bone of load-bearing region is an essential mission and effort direction of materials experts and Orthopaedics Surgeons. Current biomaterials used in load-bearing region are limited because of poor bioactivity. Therefore the generation of bioactive bone substitute with improved mechanical features is a possible way to dissolve these clinical problems. We develop a new bioactive bone substitute, Macro- Pore Bone Block (MPBB), which presents good biomechanical strength and persisting bioactivity. We implanted it into the femoral condyles of rabbits and also, cultured osteoblasts on the surface of the material *in vitro*. The MPBB presents better mechanical strength and shows a good bioactivity and appropriate degradation rate in the *in vivo* study. MPBB is also proved to promote osteoblast adhesion, proliferation and differentiation *in vitro*. Furthermore, even a clinical